



# Respiratory electron transfer pathways in plant mitochondria

Peter Schertl and Hans-Peter Braun \*

Abteilung Pflanzenproteomik, Institut für Pflanzengenetik, Leibniz Universität Hannover, Hannover, Germany

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## \*Correspondence:

Hans-Peter Braun, Institut für Pflanzengenetik, Abteilung Pflanzenproteomik, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany  
e-mail: [braun@genetik.uni-hannover.de](mailto:braun@genetik.uni-hannover.de)

The respiratory electron transport chain (ETC) couples electron transfer from organic substrates onto molecular oxygen with proton translocation across the inner mitochondrial membrane. The resulting proton gradient is used by the ATP synthase complex for ATP formation. In plants, the ETC is especially intricate. Besides the “classical” oxidoreductase complexes (complex I–IV) and the mobile electron transporters cytochrome c and ubiquinone, it comprises numerous “alternative oxidoreductases.” Furthermore, several dehydrogenases localized in the mitochondrial matrix and the mitochondrial intermembrane space directly or indirectly provide electrons for the ETC. Entry of electrons into the system occurs via numerous pathways which are dynamically regulated in response to the metabolic state of a plant cell as well as environmental factors. This mini review aims to summarize recent findings on respiratory electron transfer pathways in plants and on the involved components and supramolecular assemblies.

**Keywords:** plant mitochondria, electron transport chain, dehydrogenase, alternative oxidase, respiratory supercomplex

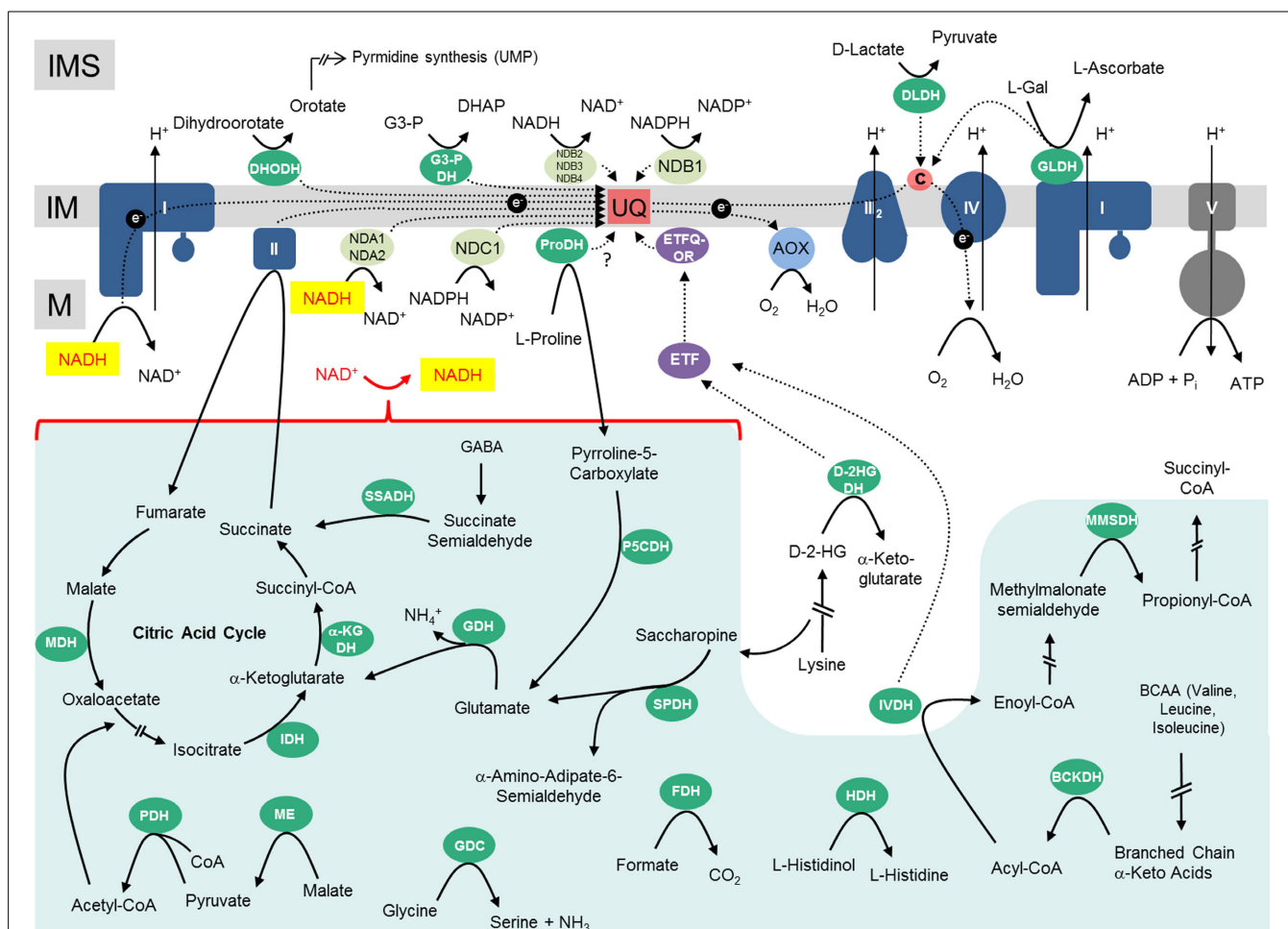
## INTRODUCTION

During cellular respiration, organic compounds are oxidized to generate usable chemical energy in the form of ATP. The respiratory electron transport chain (ETC) of mitochondria is at the center of this process. Its core consists of four oxidoreductase complexes, the NADH dehydrogenase (complex I), the succinate dehydrogenase (complex II), the cytochrome c reductase (complex III) and the cytochrome c oxidase (complex IV), as well as of two mobile electron transporters, cytochrome c, and the lipid ubiquinone. Overall, electrons are transferred from the coenzymes NADH or FADH<sub>2</sub> onto molecular oxygen which is reduced to water. Three of the four oxidoreductase complexes (complexes I, III and IV) couple their electron transfer reactions with proton translocation across the inner mitochondrial membrane. As a result, a proton gradient is formed which can be used by the ATP synthase complex (complex V) for the phosphorylation of ADP. In its classically described form, cellular respiration is based on a linear ETC (from NADH via complexes I, III, and IV to molecular oxygen). However, electrons can enter and leave the ETC at several alternative points. This is especially true for the plant ETC system, which is highly branched. In this review we aim to integrate current knowledge on the ETC system in plants with respect to its components, electron transport pathways and supramolecular structure.

## COMPONENTS OF THE PLANT ETC SYSTEM

The “classical” oxidoreductase complexes of the respiratory chain (given in dark blue in **Figure 1**) resemble their homologues in animal mitochondria but at the same time have some clear distinctive features (reviewed in Millar et al., 2008, 2011; Rasmusson and Moller, 2011; van Dongen et al., 2011; Jacoby et al., 2012). *Complex I* is especially large in plant mitochondria and includes

nearly 50 different subunits (Braun et al., 2014). Compared to its homologs from bacteria and other eukaryotic lineages it has an extra domain which includes carbonic anhydrase-like proteins. The function of this additional domain is currently unclear but it has been suggested to be important in the context of an intercellular CO<sub>2</sub> transfer mechanism to provide mitochondrial CO<sub>2</sub> for carbon fixation in chloroplasts (Braun and Zabaleta, 2007; Zabaleta et al., 2012). *Complex II* is composed of four subunits in bacteria and mitochondria of animals and fungi. In plants complex II includes homologs of these subunits but additionally four extra proteins of unknown function (Millar et al., 2004; Huang and Millar, 2013). In contrast, the subunit composition of *complex III* from plants is highly similar to the ones in yeast and bovine mitochondria (Braun and Schmitz, 1995a). The two largest subunits of this protein complex, termed “core proteins” in animals and fungi, are homologous to the two subunits of the mitochondrial processing peptidase (MPP) which removes pre-sequences of nuclear-encoded mitochondrial proteins after their import into mitochondria. In animal mitochondria, the core proteins are proteolytically inactive. Instead, an active MPP is present within the mitochondrial matrix. In contrast, the core subunits of complex III from plants have intact active sites (Braun et al., 1992; Glaser et al., 1994). Indeed, complex III isolated from plant mitochondria efficiently removes pre-sequences of mitochondrial pre-proteins. The differing functional states of complex III in diverse eukaryotic lineages might reflect different evolutionary stages of this protein complex (Braun and Schmitz, 1995b). Also *complex IV* has some extra subunits in mitochondria of plants (Millar et al., 2004). Eight subunits are homologous to complex IV subunits from other groups of eukaryotes and another six putative subunits represent proteins of unknown functions.



**FIGURE 1 | Mitochondrial dehydrogenases and the respiratory chain.**

Within the mitochondrial matrix (M) numerous dehydrogenases generate NADH by oxidizing various carbon compounds. NADH subsequently is re-oxidized at the inner mitochondrial membrane (IM) by the respiratory electron transfer chain (ETC). The electrons of NADH can enter the ETC through complex I or at the ubiquinone level via alternative NAD(P)H-dehydrogenases. Besides, some dehydrogenases of the mitochondrial matrix transfer electrons to ubiquinone via the ETF/ETFQOR system. Proline dehydrogenase possibly directly transfers electrons onto ubiquinone. In the intermembrane space (IMS), electrons from NAD(P)H generated in the cytoplasm can be inserted into the ETC via alternative NAD(P)H dehydrogenases. Furthermore, some dehydrogenases of the IMS can directly transfer electrons onto ubiquinone or cytochrome c. Color code—dark blue, protein complexes of the ETC; blue, AOX; purple, ETF/ETFQOR system; light green, alternative NAD(P)H dehydrogenases of the ETC; green, dehydrogenases; red, ubiquinone and cytochrome c; yellow, NADH produced by dehydrogenases of the mitochondrial matrix/NADH re-oxidized by complex I or internal alternative NADH dehydrogenases; dark gray, ATP synthase complex; light green background, NADH producing

dehydrogenases of the mitochondrial matrix. Abbreviations—*alphabetically ordered*. I, complex I; II, complex II; III, complex III; IV, complex IV; V, complex V;  $\alpha$ -KGDH,  $\alpha$ -ketoglutarate dehydrogenase; AOX, alternative oxidase; BCKDH, branched-chain  $\alpha$ -ketoacid dehydrogenase complex; c, cytochrome c; D-2HGDH, D-2-hydroxyglutarate dehydrogenase; DHODH, dihydroorotate dehydrogenase; DLDH, D-lactate dehydrogenase; ETF, electron transfer flavoprotein; ETFQOR, electron transfer flavoprotein ubiquinone oxidoreductase; FDH, formate dehydrogenase; GDC, glycine dehydrogenase; GDH, glutamate dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; G3-PDH, glyceraldehyde 3-phosphate dehydrogenase; HDH, histidinol dehydrogenase; IDH, isocitrate dehydrogenase; IVDH, isovaleryl-coenzyme A dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; MMSDH, methylmalonate-semialdehyde dehydrogenase; NDA1/2, NDB2/3/4, alternative NADH dehydrogenase; NDC1, NDB1, alternative NADPH dehydrogenase; P5CDH, pyrroline-5-carboxylate dehydrogenase; PDH, pyruvate dehydrogenase; ProDH, proline dehydrogenase; SPDH, saccharopine dehydrogenase; SSADH, succinic semialdehyde dehydrogenase; UQ, ubiquinone. For further information of the enzymes see **Table 1**.

The ETC of plant mitochondria additionally includes several so-called “alternative oxidoreductases”: the alternative oxidase (AOX; light blue in **Figure 1**) and several functionally distinguishable alternative NAD(P)H dehydrogenases (alternative NDs, light green in **Figure 1**). Findings on their functional roles have been reviewed recently (Rasmusson et al., 2008; Rasmusson and Moller, 2011; Moore et al., 2013). AOX directly transfers

electrons from ubiquinol to molecular oxygen and therefore constitutes an alternative electron exit point of the ETC. As a result, complexes III and IV are excluded from respiratory electron transport. The alternative NAD(P)H dehydrogenases serve as alternative electron entry points of the plant ETC and may substitute complex I. They differ with respect to co-factor requirement and localization at the outer or inner surface of

**Table 1 | Mitochondrial dehydrogenases in *Arabidopsis thaliana*<sup>a</sup>.**

Enzyme	Accession no. <sup>b</sup> subunits isoforms etc.	Catalysed reaction	Oligomeric state Native mass/monomer mass according to GelMap <sup>c</sup> (according to other data in the literature)	Publication <sup>d</sup> for <i>Arabidopsis</i> (for other plants)
Malate dehydrogenase	At1g53240 At3g15020	Malate + NAD <sup>+</sup> ⇌ Oxaloacetate + NADH	At1g53240: 89 kDa/42 kDa At3g47520: 157 kDa/38 kDa	Journet et al., 1981 Gietl, 1992 Krömer, 1995 Nunes-Nesi et al., 2005 Lee et al., 2008 Tomaz et al., 2010
Isocitrate dehydrogenase	At4g35260 At5g14590 At4g35650 At3g09810 At5g03290 At2g17130	Isocitrate + NAD <sup>+</sup> ⇌ α-Ketoglutarate + CO <sub>2</sub> + NADH	At4g35260: 89 kDa/42 kDa At5g14590: 140 kDa/53 kDa At3g09810: 138 kDa/40 kDa At5g03290: 138 kDa/40 kDa	Behal and Oliver, 1998 Lancien et al., 1998 Lin et al., 2004 Lemaitre and Hodges, 2006 Lemaitre et al., 2007
α-Ketoglutarate dehydrogenase complex	At3g55410 (E1) At5g65750 (E1) At4g26910 (E2) At5g55070 (E2) At3g17240 (E3) At1g48030 (E3) At3g13930 (E3)	α-Ketoglutarate + coenzyme A + NAD <sup>+</sup> ⇌ succinyl-CoA + CO <sub>2</sub> + NADH	At5g65750: 207 kDa/91 kDa At3g55410: 207 kDa/91 kDa  (1.7 MDa complex)	Poulsen and Wedding, 1970 Wedding and Black, 1971a,b Dry and Wiskich, 1987 Millar et al., 1999 Araújo et al., 2008 Araújo et al., 2013
Glutamate dehydrogenase	At5g18170 At5g07440 At3g03910	Glutamate + H <sub>2</sub> O + NAD <sup>+</sup> ⇌ α-Ketoglutarate + NH <sub>4</sub> <sup>+</sup> + NADH	At5g18170: 209 kDa/48 kDa At5g07440: 209 kDa/48 kDa At3g03910: 209 kDa/48 kDa	Yamaya et al., 1984 Turano et al., 1997 Aubert et al., 2001 Miyashita and Good, 2008a,b Fontaine et al., 2012 Tarasenko et al., 2013 Fontaine et al., 2012
Malic enzyme	At2g13560 At4g00570 At1g79750	Malate + NAD <sup>+</sup> ⇌ Pyruvate + NADH + CO <sub>2</sub>	At2g13560: 370 kDa/63 kDa At4g00570: 370 kDa/63 kDa	Jenner et al., 2001 Tronconi et al., 2008 Tronconi et al., 2010 Tronconi et al., 2012
Pyruvate dehydrogenase complex	At1g59900 (E1) At1g24180 (E1) At5g50850 (E1) At3g52200 (E2) At1g54220 (E2) At3g13930 (E3) At3g17240 (E3) At1g48030 (E3)	Pyruvate + coenzyme A + NAD <sup>+</sup> ⇌ Acetyl-CoA + CO <sub>2</sub> + NADH	At3g13930: 1500 kDa/54 kDa At1g24180: 470 kDa/41 kDa At5g50850: 150 kDa/39 kDa At1g59900: 138 kDa/44 kDa  (9.5 MDa complex)	Luethy et al., 1994 Grof et al., 1995 Zou et al., 1999 Tovar-Méndez et al., 2003 Szurmak et al., 2003 Yu et al., 2012
Glycine dehydrogenase complex	At4g33010 (P) At2g26080 (P) At1g32470 (H) At2g35120 (H) At2g35370 (H) At1g11860 (T) At4g12130 (T) At3g17240 (L) At1g48030 (L)	Glycine + H <sub>4</sub> folate + NAD <sup>+</sup> ⇌ methylene-H <sub>4</sub> folate + CO <sub>2</sub> + NH <sub>3</sub> + NADH	At4g33010: 144 kDa/91 kDa At2g26080: 209 kDa/91 kDa At1g11860: 148 kDa/46 kDa  (1.3 MDa complex)	Somerville and Ogren, 1982 Oliver et al., 1990 Oliver, 1994 Srinivasan and Oliver, 1995 Douce et al., 2001

(Continued)

**Table 1 | Continued**

<b>Enzyme</b>	<b>Accession no.<sup>b</sup></b> subunits isoforms etc.	<b>Catalysed reaction</b>	<b>Oligomeric state</b> Native mass/monomer mass according to GelMap <sup>c</sup> (according to other data in the literature)	<b>Publication<sup>d</sup></b> for Arabidopsis (for other plants)
Branched-chain alpha keto acid dehydrogenase complex	At5g09300 (E1) At1g21400 (E1) At1g55510 (E1) At3g13450 (E1) At3g06850 (E2) At3g13930 (E3) At3g17240 (E3) At1g48030 (E3)	Branched chain alpha keto-acids + CoA + NAD <sup>+</sup> ⇌ Acyl-CoA + NADH	At1g55510: 150 kDa/39 kDa  (0.95 MDa complex)	Fujiki et al., 2000 Mooney et al., 2000 Fujiki et al., 2001 Fujiki et al., 2002 Taylor et al., 2004 Binder, 2010
Formate dehydrogenase	At5g14780	Formate + NAD <sup>+</sup> ⇌ CO <sub>2</sub> + NADH	(200 kDa complex)	Halliwell, 1974 Colas des Francs-Small et al., 1993 Hourton-Cabassa et al., 1998 Jansch et al., 1996 Bykova et al., 2003 Baack et al., 2003 Olson et al., 2000 Alekseeva et al., 2011
Methylmalonate semialdehyde dehydrogenase	At2g14170	(S)-methylmalonate- semialdehyde + coenzyme A + NAD <sup>+</sup> + H <sub>2</sub> O ⇌ propanoyl-CoA + bicarbonate + NADH	At2g14170: 200 kDa/59 kDa	Oguchi et al., 2004 Tanaka et al., 2005 Kirch et al., 2004
Isovaleryl-CoA dehydrogenase	At3g45300	Isovaleryl-CoA + acceptor (ETF) ⇌ 3-methylbut-2-enoyl-CoA + reduced acceptor (ETF) (also considerable activity with other acyl-CoAs)	At3g45300: 132 kDa/46 kDa  (homodimeric complex)	Däschner et al., 1999 Reinard et al., 2000 Faivre-Nitschke et al., 2001 Däschner et al., 2001 Goetzman et al., 2005 Araújo et al., 2010
D-2-Hydroxyglutarate dehydrogenase	At4g36400	D-2-hydroxyglutarate + acceptor (ETF) ⇌ 2-oxoglutarate + reduced acceptor (ETF)	(homodimeric complex)	Engqvist et al., 2009 Araújo et al., 2010 Engqvist et al., 2011
Saccharopine dehydrogenase	At5g39410	Saccharopine + NAD <sup>+</sup> + H <sub>2</sub> O ⇌ Glutamate + -Amino adipate semialdehyde + NADH	not known	Zhu et al., 2000 Heazlewood et al., 2003
Pyrroline-5- carboxylate dehydrogenase	At5g62530	Pyrroline-5-carboxylate + NAD <sup>+</sup> ⇌ Glutamate (Glutamate-5- semialdehyde) + NADH	At5g62530: 158 kDa/59 kDa	Forlani et al., 1997 Deuschle et al., 2001 Deuschle et al., 2004 Miller et al., 2009
Proline dehydrogenase	At3g30775 At5g38710	L-Proline ⇌ Pyrroline-5-Carboxylate	not known	Elthon and Stewart, 1981 Verbruggen et al., 1996 Kiyosue et al., 1996 Mani et al., 2002 Szabados and Savouré, 2010 Funck et al., 2010 Sharma and Verslues, 2010 Schertl et al., in press

(Continued)

**Table 1 | Continued**

Enzyme	Accession no. <sup>b</sup> subunits isoforms etc.	Catalysed reaction	Oligomeric state Native mass/monomer mass according to GelMap <sup>c</sup> (according to other data in the literature)	Publication <sup>d</sup> for Arabidopsis (for other plants)
L-Galactono-1,4-lactone dehydrogenase	At3g47930	L-Galactono-1,4-Lactone $\rightleftharpoons$ L-Ascorbate	(420 kDa, 470 kDa, 850 kDa complexes)	Mapson and Breslow, 1958 Siendones et al., 1999 Leferink et al., 2008 Pineau et al., 2008 Leferink et al., 2009 Schertl et al., 2012
D-Lactate dehydrogenase	At5g06580	D-Lactate $\rightleftharpoons$ Pyruvate	(homodimeric complex)	Bari et al., 2004 Atlante et al., 2005 Engqvist et al., 2009 Wienstroer et al., 2012
Glycerol-3-phosphate dehydrogenase	At3g10370	Glycerol 3-phosphate $\rightleftharpoons$ Dihydroxyacetonephosphate	At3g10370: 160 kDa/65 kDa	Shen et al., 2003 Shen et al., 2006
Dihydroorotate dehydrogenase	At5g23300	Dihydroorotate $\rightleftharpoons$ Orotate	At5g23300: 156 kDa/49 kDa	Ullrich et al., 2002 Doremus and Jagendorf, 1985 Miersch et al., 1987
Succinic semialdehyde dehydrogenase	At1g79440	Succinic semialdehyde $\rightleftharpoons$ Succinate	At1g79440: 163 kDa/55 kDa	Busch and Fromm, 1999 Bouché et al., 2003 Kirch et al., 2004 Toyokura et al., 2011
Histidinol dehydrogenase	At5g63890	L-histidinol + NAD <sup>+</sup> $\rightleftharpoons$ L-histidine + NADH	At5g63890: 115 kDa/51 kDa	Nagai and Scheidegger, 1991 Ingle, 2011
Alternative NAD(P)H dehydrogenases (NDA1, NDB4, NDA2, NDB2, NDB3, NDB1, NDC1)	At1g07180 At2g20800 At2g29990 At4g05020 At4g21490 At4g28220 At5g08740	NAD(P)H + UQ $\rightleftharpoons$ NAD(P) <sup>+</sup> + UQH <sub>2</sub>	At2g20800: 160 kDa/65 kDa At2g29990: 163 kDa/55 kDa At4g05020: 160 kDa/65 kDa	Escobar et al., 2004 Rasmusson et al., 2004 Rasmusson et al., 2008 Wulff et al., 2009 Wallström et al., 2014a,b

<sup>a</sup> Mitochondrial dehydrogenases without complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) of the respiratory chain. This list corresponds to the dehydrogenases shown in **Figure 1**.

<sup>b</sup> Accession numbers in accordance with The Arabidopsis Information Resource (TAIR).

<sup>c</sup> Oligomeric state: native mass and monomer mass according to GelMap (<https://gelmap.de/231>).

<sup>d</sup> Key publications for Arabidopsis (other plants).

the inner mitochondrial membrane (external alternative NDs, internal alternative NDs). Some of the genes encoding alternative NDs are activated by light (Rasmusson et al., 2008; Rasmusson and Moller, 2011). The latter enzymes are considered to be important during photorespiration and all alternative enzymes during various stress conditions. Since none of the alternative oxidoreductases couple electron transfer with proton translocation across the inner mitochondrial membrane, their enzymatic function is believed to be important in the context of an overflow protection mechanism for the ETC which is especially relevant during high-light conditions.

Finally, dehydrogenases (dark green in **Figure 1**; **Table 1**) can directly or indirectly insert electrons into the respiratory chain (Rasmusson et al., 2008; Rasmusson and Moller, 2011). Numerous dehydrogenases of the mitochondrial matrix

generate NADH which is re-oxidized by complex I and the internal alternative NDs. However, some dehydrogenases directly transfer electrons onto ubiquinone [dihydroorotate dehydrogenase (DHODH), glyceraldehyde 3-phosphate dehydrogenase (G3-PDH) and possibly proline dehydrogenase (ProDH)] or onto cytochrome c [L-galactone-1,4-lactone dehydrogenase (GLDH) and D-lactate dehydrogenase (DLDH)]. Furthermore, at least two dehydrogenases [isovaleryl-coenzyme A dehydrogenase (IVDH) and D-2-hydroxyglutarate dehydrogenase (D-2HGDH)] transfer electrons onto ubiquinone via a short electron transfer chain composed of the “electron transfer flavoprotein” and the “electron transfer flavoprotein-ubiquinone oxidoreductase” (ETF and ETFQ-OR, purple in **Figure 1**) (Ishizaki et al., 2005, 2006; Araújo et al., 2010). IVDH is involved in the branched chain amino acid catabolism and D-2HGDH in the catabolism of lysine. In



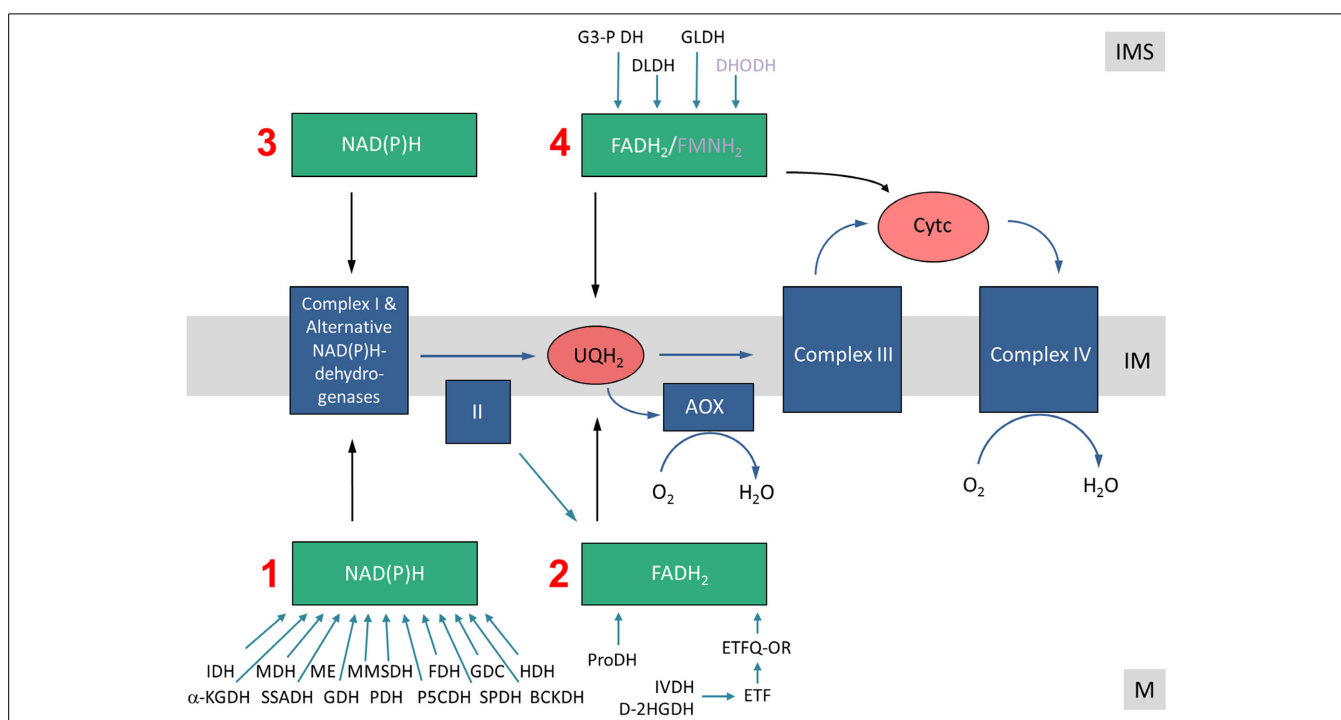
plants, degradation of amino acids for respiration was shown to be especially important during carbon starvation conditions, e.g., extended darkness (Araújo et al., 2011). In contrast to animal mitochondria, fatty acid oxidation does not take place in plant mitochondria and the involved dehydrogenases consequently are absent. Instead, additional metabolic pathways occur in plants, e.g., the final step of an ascorbic acid biosynthesis pathway, which is catalyzed by GLDH. Electrons of L-galactono-1,4-lactone (GL) oxidation are inserted into the ETC via cytochrome c (Bartoli et al., 2000). Proline, besides being a building block for protein biosynthesis, is used as an osmolyte in plant cells. Proline is catabolized in mitochondria by a two-step process involving pyrroline-5-carboxylate dehydrogenase (P5CDH) and ProDH (Szabados and Savouré, 2010). P5CDH produces NADH, whereas ProDH represents a flavoenzyme which is assumed to transfer electrons directly or indirectly onto ubiquinone. Some additional dehydrogenases occur in plant mitochondria in the mitochondrial matrix and the intermembrane space

which also contribute electrons to the ETC (Figure 1, Table 1). However, in some cases their mitochondrial localization is not entirely certain and should be further investigated by future research.

### ELECTRON ENTRY PATHWAYS INTO THE ETC

All electrons enter the ETC via NAD(P)H (generated by a variety of dehydrogenases in the mitochondrial matrix or the intermembrane space/the cytoplasm) or via flavine nucleotides (FADH<sub>2</sub>, FMNH<sub>2</sub>), which generally are bound to proteins termed flavoproteins. Consequently, the following electron entry pathways into the ETC can be defined: (i) the Matrix NAD(P)H pathway, (ii) the Matrix-FADH<sub>2</sub> pathway, (iii) the Intermembrane-space-NAD(P)H pathway, and (iv) the Intermembrane-space-FADH<sub>2</sub>/FMDH<sub>2</sub> pathway (Figure 2).

Different metabolic processes, which vary depending on the physiological state of the plant cell, contribute to the four electron entry pathways. During stable carbohydrate conditions, electrons



**FIGURE 2 | Electron entry pathways into the mitochondrial electron transport chain in plants.** Electrons enter the respiratory chain via four different pathways. (1) The Matrix-NAD(P)H pathway. Various dehydrogenases oxidize carbon compounds in the mitochondrial matrix. Electrons are transferred in the form of NADH to the ETC. NADH is re-oxidized by complex I or the internal alternative NAD(P)H dehydrogenases. (2) The Matrix-FADH<sub>2</sub> pathway. FAD-containing enzymes oxidize carbon compounds in the mitochondrial matrix and directly (ProDH?) or indirectly (via the ETF/ETFQ system) transfer electrons to the ubiquinol pool. (3) The IMS-NAD(P)H pathway. Cytoplasmically formed NAD(P)H is re-oxidized via external alternative dehydrogenases. (4) The IMS-FADH<sub>2</sub> pathway. FAD/FMN-containing enzymes oxidize carbon compounds in the mitochondrial intermembrane space. Electrons are transferred either to the ubiquinol or the cytochrome c. M, matrix; IM, inner membrane; IMS, intermembrane space. Abbreviations—alphabetically ordered. I,

complex I; II, complex II; III, complex III; IV, complex IV; α-KGDH, α-ketoglutarate dehydrogenase; AOX, alternative oxidase; BCKDH, branched-chain α-ketoacid dehydrogenase complex; Cyt c, cytochrome c; D-2HGDH, D-2-hydroxyglutarate dehydrogenase; DHODH, dihydroorotate dehydrogenase; DLDH, D-lactate dehydrogenase; ETF, electron transfer flavoprotein; ETFQOR, electron transfer flavoprotein ubiquinone oxidoreductase; FDH, formate dehydrogenase; GDC, glycine dehydrogenase; GDH, glutamate dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; G3-PDH, glyceraldehyde 3-phosphate dehydrogenase; HDH, histidinol dehydrogenase; IDH, isocitrate dehydrogenase; IVDH, isovaleryl-coenzyme A dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; MMSDH, methylmalonate-semialdehyde dehydrogenase; P5CDH, pyrroline-5-carboxylate dehydrogenase; PDH, pyruvate dehydrogenase; ProDH, proline dehydrogenase; SPDH, saccharopine dehydrogenase; SSADH, succinic semialdehyde dehydrogenase; UQH<sub>2</sub>, ubiquinol.

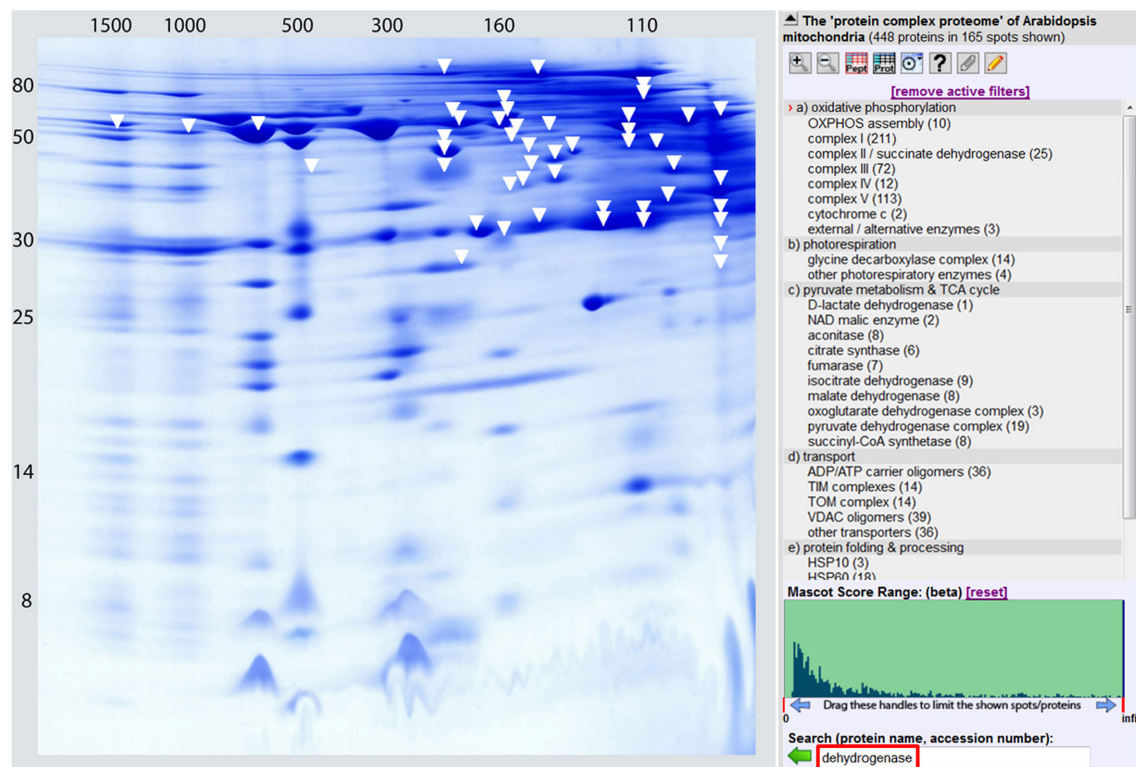
for the respiratory chain can be supplied by NADH and FADH<sub>2</sub> provided by the tricarboxylic acid (TCA) cycle. This is believed to be the standard mode of cellular respiration in non-green plant tissues or green tissues at night and resembles the basic situation in animal cells. However, during photosynthesis, NADH generation of the TCA cycle is reduced because some of its intermediates are used for anabolic reactions (reviewed in Sweetlove et al., 2010). Furthermore, the pyruvate dehydrogenase (PDH) complex is deactivated in plant mitochondria in the light by phosphorylation (Budde and Randall, 1990). At the same time photorespiration leads to an increase in NADH formation in the mitochondrial matrix by the activity of the glycine dehydrogenase complex (GDC). Indeed, at high-light conditions, NADH formed by GDC is believed to be the main substrate of the ETC, and not the NADH formed by the enzymes of the TCA cycle. At the same time, plant cells might become over-reduced in the presence of high-light. In this situation alternative oxidoreductases can insert excess electrons into the respiratory chain without contributing to the proton gradient. Upon carbon starvation conditions (e.g., extended darkness) electrons from the breakdown of amino acids are provided to the ETC (Araújo et al., 2011). Especially after release of salt stress the amino acid proline is used as an electron source (Szabados and Savaouré, 2010). In summary, electron entry into the ETC is a highly flexible process in plants

which much depends on light, the metabolic state of the cell as well as environmental stress factors.

### SUPRAMOLECULAR STRUCTURE OF THE ETC SYSTEM

The ETC is based on defined protein-protein interactions. Most stable interactions occur within the four “classical” oxidoreductase complexes of the respiratory chain. Indeed, complexes I to IV can be isolated in intact form by various biochemical and electrophoretic procedures. Furthermore, several lines of evidence indicate that complexes I, III and IV interact within the inner mitochondrial membrane forming respiratory supercomplexes (reviewed in Dudkina et al., 2008). Complex I as well as complex IV associate with dimeric complex III (I + III<sub>2</sub> and IV<sub>2</sub> + III<sub>2</sub> supercomplexes). An even larger supercomplex includes complexes I, III<sub>2</sub>, and IV and was proposed to be called “respirasome” because it can autonomously catalyzes the overall ETC reaction in the presence of ubiquinone and cytochrome c. The alternative oxidoreductases of the plant ETC seem not to be part of the respiratory supercomplexes. However, alternative NDs were found to be part of other protein complexes of about 160 kDa (Klodmann et al., 2011) or 150–700 kDa (Rasmusson and Agius, 2001).

Experimental data also indicate that several of the mitochondrial dehydrogenases form protein complexes. TCA cycle



**FIGURE 3 | The dehydrogenase subproteome of plant mitochondria.**

Mitochondrial proteins from *Arabidopsis thaliana* were separated by 2D Blue native/SDS PAGE and displayed via GelMap (<https://gelmap.de/231#>). Protein separation under native condition was from left to right, protein separation in the presence of SDS from top

to bottom. Molecular masses of standard proteins are given to the left/above the 2D gel. All proteins annotated as “dehydrogenase” are indicated by white arrows. Exception: The subunits of complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) are not indicated on the figure.

enzymes can assemble forming multienzyme clusters (Barnes and Weitzman, 1986). In addition, some of the mitochondrial dehydrogenases interact with ETC complexes, e.g., malate dehydrogenase has been reported to interact with complex I in animal mitochondria (Fukushima et al., 1989; see Braun et al., 2014 for review). Information on the native state of mitochondrial dehydrogenases furthermore comes from the GelMap project (Klodmann et al., 2011). Using 2D Blue native/SDS PAGE and systematic protein identifications, various dehydrogenases were described (Figure 3, Table 1). Native molecular mass of the dehydrogenases in many cases much exceeds the molecular mass of the monomeric proteins (Table 1, column 3). This indicates that probably most mitochondrial dehydrogenases form part of defined higher order structures.

## CONCLUSION AND OUTLOOK

Cellular respiration in plants is an especially dynamic system. The classical protein complexes of the ETC have extra functions and several alternative oxidoreductases occur. A network of mitochondrial dehydrogenases directly or indirectly supplies electrons for the respiratory chain. Insertion of electrons via various pathways is highly dependent on the metabolic state of the plant cell. The regulation of electron entry pathways into the respiratory chain is only partially understood and might, besides others, depend on the formation of supramolecular structures. Non-invasive experimental procedures will be necessary to physiologically investigate the function of these structures by future research.

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